sufficiently slow that the metabolic rate of sperm is decreased, to provide a cooled [sperm] first sample solution, maintained for a period of time at said first temperature;

b) adding a solution comprising glycerol to [the] said cooled [sperm] first sample solution; [and]

c) mixing said glycerol and said cooled first sample solution together to comprise a second sample solution;

[c)] d) lowering the temperature of [freezing] said [cooled sperm] second sample solution to a second temperature for a sufficient period of time to equilibrate glycerol and sperm; and

e) freezing said second sample solution to thereby provide a frozen sperm sample, such that the sperm is preserved[.];

claim 2 → wherein said first sample solution does not contain glycerol;

new limitation → wherein said second sample solution further comprises an antibiotic compound;

claim 1 → wherein said sperm sample is cooled to said first temperature and maintained at said first temperature for 4 hours to 21 hours;

wherein said second sample solution is allowed to freeze at said second temperature.

A1

Please Cancel Claim 2

3. (Amended) The method of claim 1, wherein [the] said second sample solution [cryoprotectant buffer] comprises about 10% to about 30% egg yolk.

4. (Amended) The method of claim 1, wherein [the] said second sample solution comprises [concentration of glycerol in the sample after the addition of the glycerol solution is about] 5% to about 10% glycerol.

5. (Amended) The method of claim 1, wherein [the] said sperm sample is obtained from a mammal.

6. (Amended) The method of claim 1, wherein [the] said first temperature is between [about] 0°C and [to about] 10°C.

Please Cancel Claim 7

8. (Amended) The method of claim 1, wherein [the sperm] said first sample solution is cooled at a rate of between 0.2°C [to about] and 0.5°C per minute to [the] reach said first temperature.

9. (Amended) The method of claim 1, wherein [the sperm] said first sample solution is cooled to the first temperature over the course of [about] 1.5 to [about] 4 hours.

10. (Amended) The method of claim 1, wherein [the] said second temperature is between [about] -40°C [to about] and -100°C.

11. (Amended) The method of claim 1, wherein the sample is maintained at [the] said second temperature for between [about] 7 minutes and [to about] 20 minutes.

12. (Amended) The method of claim 1, wherein the method further comprises storing the frozen sperm sample at a third temperature of [about] between -190°C [to about] and -200°C.

13. (Amended) A method of preserving sperm comprising:

a) combining a sperm sample with a first cryoprotectant buffer to form a first cryoprotectant solution;

b) cooling [sperm] said first cryoprotectant solution to a first temperature such first temperature being between [about] 2°C [to about] and 10°C at a rate sufficiently slow that the metabolic rate of sperm is decreased to produce cooled sperm;

c) lowering the temperature of said first cryoprotectant solution to [the cooled sperm at] a second temperature between [about] -60°C and [to about] -90°C wherein said first cryoprotectant solution can freeze; and

d) storing [the frozen sperm] said frozen first cryoprotectant solution in liquid nitrogen at a third temperature[.];

wherein said sperm sample is cooled to said first temperature and maintained at said first temperature for 4 hours to 21 hours;

wherein said first cryoprotectant solution further comprises an antibiotic compound.

14. (Amended) The method of claim 13, wherein [the] said first cryoprotectant buffer comprises [about] 5% to [about] 10% glycerol weight to volume of said cryoprotectant buffer.

Please Cancel Claim 15

16. (Amended) The method of claim 13, wherein [the] said sperm sample is obtained from a mammal.

17. (Amended) The method of claim 13, wherein [the] said sperm sample is maintained at [the] said first temperature for between [about] 4 hours [to about] and 21 hours.

18. (Amended) The method of claim 13, wherein [the] said [sperm] first cryoprotectant solution is cooled at a rate of between [about] 0.2°C and [to about] 0.5°C per minute to reach said [the] first temperature.

19. (Amended) The method of claim 13, wherein [the sperm] said first cryoprotectant solution is cooled to [the] said first temperature over the course of [about] 1.5 hours to [about] 4 hours.

20. (Amended) The method of claim 13, wherein a second cryoprotectant buffer is added to said first cryoprotectant solution [the sample] after [the] said first cryoprotectant solution has been [sperm is] cooled to [the] said first temperature, [and] but before [the sperm is] said first cryoprotectant solution has been further cooled to [the] said second temperature or frozen.

21. (Amended) The method of claim 20 wherein [the] said second cryoprotectant buffer comprises [about] 5% to [about] 10% glycerol.

22. (Amended) The method of claim 13, wherein [the] said second temperature is at least [about] -80°.

23. (Amended) The method of claim 13, wherein said first cryoprotectant solution [the sample] is maintained at [the] said second temperature for between [about] 7 minutes [to about] and 20 minutes.

24. (Amended) A method of [providing] preserving sperm comprising:

a) providing a sample comprising sperm;

b) isolating sperm from [the] said sample;

c) combining said isolated sperm with a first cryoprotectant buffer to form a first sample solution [which lacks glycerol];

d) cooling said [sperm] first sample solution to a first temperature [of about] between 2°C [to about] and 8°C at a rate of [about] between 0.2°C and [to] 0.5°C per minute to produce [cooled sperm] said first sample solution at said first temperature;

e) adding a second cryoprotectant buffer [which contains] comprising glycerol to form a second sample solution;

f) maintaining [the cooled sperm at the first temperature] said second sample solution at said first temperature for a [duration] period of between [about] 4 hours [to about] and 21 hours;

g) [freezing the cooled sperm] lowering the temperature of said second sample solution by cooling said second sample solution to [at] a second temperature of [about] between -60°C [to about] and -90°C for a time of between about 10 minutes to about 15 minutes;

h) allowing said second sample solution to freeze at said second temperature;

[h)] i) storing [the frozen sperm] said second sample solution at a third temperature of between [about] -180°C [to about] and -220°C for a desired time period; [and]

i) thawing said sperm, to thereby provide sperm for use [.]; and

wherein said first sample solution does not contain glycerol;

wherein said second sample solution further comprises an antibiotic compound.

25. (Amended) The method of claim 24, wherein the sperm are thawed for about 90 seconds in a water bath at about 37°C prior to use.

26. (Amended) A method of making an animal, comprising fertilizing an oocyte with sperm preserved by the method of claims 1, [claim] 13, or [claim] 24.

Please add claims 27 – 44

27. (New) A method of preserving sperm comprising:

a) cooling a first sample solution which includes sperm to a first temperature sufficient to protect sperm from glycerol toxicity, at a rate sufficiently slow

that the metabolic rate of sperm is decreased, to provide a cooled first sample solution, maintained for a period of time at said first temperature;

b) adding a cryoprotectant solution to said cooled first sample solution;

c) mixing said cryoprotectant solution and said cooled first sample solution together to comprise a second sample solution;

d) lowering the temperature of said second sample solution to a second temperature for a sufficient period of time to equilibrate said cryoprotectant solution and sperm comprising said second sample solution; and

e) freezing said second sample solution to thereby provide a frozen sperm sample, such that the sperm is preserved;

wherein said cryoprotectant solution is further comprised of egg yolk, fructose, citric acid, Tris buffer; and an antibiotic compound;

wherein said sperm sample is cooled to said first temperature and maintained at said first temperature for 4 hours to 21 hours.

28. (New) The method of claim 27 wherein neither said first sample solution or said second sample solution contain glycerol.

29. (New) The method of claim 27 wherein said egg yolk comprises 10% to 30% weight to volume of said cryoprotectant solution.

30. (New) The method of claim 27 wherein said egg yolk comprises 20% weight to volume of said cryoprotectant solution.

31. (New) The method of claim 27 wherein said fructose comprises at least 1% weight to volume of said cryoprotectant solution.

32. (New) The method of claim 27 wherein said citric acid comprises at least 1.5% weight to volume of said cryoprotectant solution.

33. (New) The method of claim 27 wherein said antibiotic compound is selected from a group consisting of:

a) tylosin;

b) gentamicin;

c) lincospectin; and

d) spectinomycin.

34. (New) A sperm cryoprotectant solution comprising egg yolk, fructose, citric acid, Tris buffer; and an antibiotic compound, wherein said egg yolk comprises 10% to 30% weight to volume of said cryoprotectant solution; wherein said fructose comprises at least 1% weight to volume of said cryoprotectant solution; and wherein said citric acid comprises at least 1.5% weight to volume of said cryoprotectant solution.

35. (New) The sperm cryoprotectant solution of claim 34 wherein said egg yolk comprises 20% weight to volume of said cryoprotectant solution.

36. (New) The sperm cryoprotectant solution of claim 34 wherein said fructose comprises 1% weight to volume of said cryoprotectant solution.

37. (New) The sperm cryoprotectant solution of claim 34 wherein said citric acid comprises 1.5% weight to volume of said cryoprotectant solution.

38. (New) The sperm cryoprotectant solution of claim 34 wherein said antibiotic compound is selected from a group consisting of:

a) tylosin;

b) gentamicin;

c) lincospectin; and

d) spectinomycin.

39. (New) A sperm cryoprotectant solution comprising glycerol, egg yolk, fructose, citric acid, Tris buffer; and an antibiotic compound, wherein said glycerol comprises 5% to 10% weight to volume of said sperm cryoprotectant solution; wherein said egg yolk comprises 10% to 30% weight to volume of said sperm cryoprotectant solution; wherein said fructose comprises at least 1% weight to volume of said sperm cryoprotectant solution; and wherein said citric acid comprises at least 1.5% weight to volume of said sperm cryoprotectant solution.

40. (New) The sperm cryoprotectant solution of claim 39 wherein said glycerol comprises 7% weight to volume of said cryoprotectant solution.

41. (New) The sperm cryoprotectant solution of claim 39 wherein said egg yolk comprises 20% weight to volume of said cryoprotectant solution.

42. (New) The sperm cryoprotectant solution of claim 39 wherein said fructose comprises 1% weight to volume of said cryoprotectant solution.

43. (New) The sperm cryoprotectant solution of claim 39 wherein said citric acid comprises 1.5% weight to volume of said cryoprotectant solution.

44. (New) The sperm cryoprotectant solution of claim 39 wherein said antibiotic compound is selected from a group consisting of:

a) tylosin;

b) gentamicin;

c) lincospectin; and

d) spectinomycin.